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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: DiTullio et al.
ASSIGNEE: TranXenoGen, Inc.
SERIAL NUMBER: 10/771,949 EXAMINER: Schnizer, Richard A.
FILING DATE: February 2, 2004 ART UNIT: 1635
FOR: Genetic Manipulation of Spermatogonia

October 5, 2006
Boston, Massachusetts

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PAUL A. DiTULLIO UNDER 37 C.F.R §1.132

I, Paul A. DiTullio, declare and state as follows:

1. I am an inventor of the subject matter claimed in the above-referenced patent application.

2. I received a B.S. degree in biochemistry and a M.S. degree in cell biology from the University of Vermont. I have been involved in research related to transgenic animals for more than 12 years.

3. I have read the Office Action mailed on April 5, 2006 and am familiar with the Examiner's grounds of rejection of the pending claims.

4. The testes of white leghorn roosters were infused *in vivo* using the claimed methods of gene transfer. Briefly, roosters were anesthetized with a mixture of Xylazine/Ketamine and a lateral incision made after the last rib using a procedure similar to that employed for caponization. The testes, which are suspended from the dorsal body wall posterior to the lungs and ventral to the kidneys, were then visualized through the incision and injected with between 0.1ml and 0.5ml of DNA/lipid complex using a syringe and 25 gauge 3 1/2" needle. Although the ratio of DNA to lipid can be varied, the results of these experiments demonstrated that optimal gene transfer was achieved when the ranges utilized were within those stipulated by the manufacturer. For example, rooster 12150B was infused with 10ng/υl of transgene complexed with 20% LipofectAmine (Invitrogen, Carlsbad, CA). Following treatment, roosters were either allowed to reach sexual maturity in order to collect semen for transgene analysis prior to harvesting the testes or sacrificed after 1 week to directly assess the efficiency of transgene delivery. Genomic DNA for PCR was isolated from semen or testes sections using standard techniques. Flourescent in situ hybridization (FISH) was performed using transgene specific probe and designed to demonstrate integration of the transgene into the chicken genome. The results of selected rooster experiments are presented in the table below and demonstrate that the claimed methods were successfully employed to incorporate a transgene into the spermatogonia of a rooster and the production of transgenic sperm.

Analysis of Rooster Testes and Semen Samples following *in vivo* transfection with DNA.

	Age		Semen	
Rooster	At Treatment	Testes	PCR	FISH
10420P	2months	Positive	Not tested	Not tested
10841BP	2months	Negative	Not tested	Not tested
19804B	3months	Positive	Negative	Not tested
5658P	4months	Positive	Not tested	Not tested
19851B	5months	Not tested	Negative	Not tested
12150B	5months	Positive	Positive	Positive
19817B	6months	Positive	Positive	Not tested

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date 10/5/06

Paul A. DiTullio